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Activity related energy expenditure in children and adolescents with Prader–Willi syndrome

EGAH van Mil[1]*, KR Westerterp[1], ADM Kester[2], LMG Curfs[3], WJM Gerver[4], CTRM Schrander-Stumpel[3] and WHM Saris[1]

[1]*Department of Human Biology, Maastricht University, Maastricht, The Netherlands;* [2]*Department of Methodology and Statistics, Maastricht University, Maastricht, The Netherlands;* [3]*Department of Clinical Genetics, Maastricht University, Maastricht, The Netherlands; and* [4]*Department of Pediatrics, Maastricht University, Maastricht, The Netherlands*

OBJECTIVE: To measure activity related energy expenditure in Prader–Willi syndrome (PWS) corrected for body size.
SUBJECTS: 17 PWS subjects (10 females, seven males, age 7.5–19.8 y) and 17 obese controls, matched for gender and bone age.
MEAUREMENTS: Basal metabolic rate (BMR) was measured by ventilated hood and average daily metabolic rate (ADMR) was measured with doubly labelled water. Activity induced energy expenditure (AEE) was calculated as 0.9ADMR – BMR. Activity related energy expenditure was corrected for body size using the following measures: AEE per kg body weight (AEE/kg), ADMR/BMR (PAL), and the residual of the regression of ADMR on BMR (rADMR). Group differences were analyzed by analysis of covariance adjusting for bone age, fat mass (FM) and gender.
RESULTS: ADMR, AEE and PAL were lower ($P < 0.01$) in the PWS group compared with the control group (7.14 ± 1.72, 1.07 ± 0.69 and 1.33 ± 0.15 MJ/day compared with 9.94 ± 2.64, 2.56 ± 1.03 and 1.55 ± 0.12 MJ/day respectively). The variance of AEE/kg and PAL was significantly explained by gender and PWS, while AEE was additionally explained by FM. The variance of rADMR was explained by PWS and not by FM or gender.
CONCLUSION: Activity related energy expenditure is decreased in PWS compared with controls adjusted for bone age, FM and gender.
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Keywords: Prader–Willi syndrome; obesity; child; doubly labelled water; physical activity; energy; metabolism

Introduction

Prader–Willi syndrome (PWS) is known as the most common human genetic disorder linked to obesity.[1] This complex, multisystem disorder is characterized by perinatal and neonatal hypotonia, followed by a childhood obese phase.[2,3] The obesity is likely to be caused by a combination of a low energy expenditure and a high energy intake.[4,5] Eating problems, characterized by hyperphagia, and often combined with food stealing are well reported problems in PWS,[6,4] however, the actual energy intake is difficult to measure with current methods. The doubly labelled water technique offers a valid method to measure the individual's average daily metabolic rate (ADMR)[7] which consists of basal metabolic rate (BMR), activity induced energy expenditure (AEE) and diet induced thermogenesis.[8,9] In a previous report we have demonstrated that the low energy expenditure in rest as well as during sleep could be explained by a relative low fat-free mass (FFM) as one of the major components of the pathophysiological background of PWS.[10] Schoeller *et al*[5] demonstrated that the average daily metabolic rate (ADMR) in PWS patients as well as the level of physical activity were significantly lower compared with obese controls. Other investigators[11] were unable to confirm the decreased ADMR in patients with this syndrome. One of the problems of comparing physical activity between subjects with large differences in body weight is the correction for body size. The relatively low fat-free mass, resulting in a high adiposity level in PWS, is an extra complication to this matter. Measuring physical activity directly by an actometer or pedometer could provide an alternative approach to this problem and has indeed been used to measure physical activity in PWS children.[12] However because of the large variation in physical activity the investigators could not find a difference between PWS children and obese controls. Another possibility is to measure activity using the doubly labelled water method in combination with BMR, and correct the results for body size differences to test if they support a unified conclusion.[13]

As a result of contradictory reports of total energy expenditure and physical activity levels in PWS. the specific objective was to examine whether the activity related energy expenditure, corrected for body size, is different in PWS subjects compared with matched obese controls.

*Correspondence: EGAH van Mil, Department of Human Biology, Maastricht University, PO Box 616 Maastricht, 6200 MD, The Netherlands. E-mail: g.vanmil@hb.unimaas.nl
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Methods

Subjects

Seventeen PWS subjects (10 females, seven males) were recruited with the assistance of the Dutch Prader–Willi Association. The subjects were assessed according to the Holm criteria.[14] The Holm system provides a quantitative measure PWS symptoms. PWS was preferably confirmed by either a deletion on chromosome 15 or uniparental disomy. When only clinical data were available critical evaluation took place by the same clinical geneticist. The PWS subjects were gender and bone age-matched with healthy obese controls recruited from the regional public health department. Bone age was determined by assessing epiphysial maturation by the same paediatric endocrinologist using an X-ray of the mid portion of the left hand and standard growth data.[15] It is preferable to use bone age instead of calendar age in studying energy metabolism in PWS, because it provides a correction for the delay in physiological maturation of PWS subjects.[16] None of the PWS subjects were receiving hormone therapy or treatment with human GH before or during the study. Controls with endocrine causes or other secondary causes of obesity were excluded. All subjects were measured within three months during the summer. Subject characteristics are shown in Table 1. Before the start of the study the parents gave written informed consent confirmed by an oral approval of the child. The study was approved by the medical ethical committee of Maastricht university.

General outline of protocol

Procedures for energy expenditure and body composition measurements. ADMR and total body water (TBW) were measured by doubly labelled water according to the Maastricht Protocol.[7] BMR was measured by ventilated hood.

The subject and parent were invited to the laboratory at 19.00 h after a normal dinner. At 22:00 h the subject produced a urine sample to determine the background isotope level. As a last consumption before the night the subject received an orally administered mixture of ^2H_2O and $H_2{}^{18}O$. After the dosing the subject went to sleep in a respiration chamber of which the results were previously reported.[10] The following morning the subject, came out of the respiratory chamber at 6:30 h to do the first morning voiding and immediately returned to bed for BMR measurement in an adjacent room. Because the subject went not active that morning, the BMR measurement was started after lying supine for 10 min. Oxygen consumption and carbon dioxide production were measured by means of computerized open circuit ventilated hood system, for 40–50 min, when the subject was watching television. Gas analyses were performed using a paramagnetic oxygen analyzer (Servomex, Crowborough, UK) and an infrared carbon dioxide analyzer (Uras 3G, Hartmann & Braun, Frankfurt, Germany). BMR was calculated according to Weir[17] over the 14 min interval with the lowest standard deviation. The same morning a urine sample was taken from the second voiding 10 h after dose administration. Isotope abundance in the urine was determined with an isotope-ratio mass spectrometer (Aqua Sira, VG Isogas Ltd., Micromass, Manchester, UK). TBW was calculated as the ^2H dilution space divided by 1.04, correcting for exchange of the ^2H label with non-aqueous H of body solids.[18] Fat-free mass (FFM) was assessed with the assumption of FFM containing all body water. Hydration factors of FFM were based on gender and maturation specific values.[19] Maturation was assessed according to Tanner's puberty ratings.[20] Fat mass (FM) was calculated by subtracting FFM of the subjects total body weight. Before the subjects consumed any food or drink, after voiding and whilst wearing under-clothing, body weight was measured on an electronic scale (El200, Mettler Instrument AG, Greifensee, Switzerland). Height of subjects without shoes was measured using a stadiometer. Isotope disappearance rate in the urine from the samples of days 1, 8 and 14 from the following 14 days was used to calculate carbon dioxide production. Carbon dioxide production was converted to ADMR with a

Table 1 Subject characteristics

	PWS (n=17)			*Obese controls (n=17)*		
	Mean	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*
Bone age (y)	12.7	2.9	6.9–16.0	12.7	3.2	5.6–16.0
Age (y)	11.9	3.4	7.5–19.8	11.3	2.6	6.3–15.3
Height (m)	1.43	0.16	1.15–1.65	1.49	0.20	1.1–1.72
Weight (kg)	50.0	19.7	20.1–87.8	61.5	25.6	16.1–108.0
BMI (kg/m^2)	23.5	6.0	15.2–38.1	26.0	6.5	13.5–39.4
%RBW (%)	142	30	100–224	148	29	89–204
FFM (kg)	27.5*	9.9	12.3–42.7	35.9	13.4	12.6–58.2
FM (kg)	22.4	11.7	7.8–48.0	25.6	12.7	2.8–49.8
%FM (%)	43.7	7.9	29.4–59.5	39.1	8.8	16.3–46.7

PWS, Prader–Willi syndrome; %RBW, percentage relative body weight; FFM, fat-free mass; FM, fat mass, %FM, percentage fat mass.
*Significantly different from control group (independent-samples *t*-test): $P < 0.05$.

respiratory exchange ratio (RER) equal to the food quotient (FQ) that was derived from a 1-week food diary. The weighed dietary record was handed to the parent(s) and subject, alter instruction on how to measure portion size. They were asked to record brand names, methods of preparation, and ingredients of mixed dishes. The same dietitian reviewed the record with the parent(s) and subject and calculated the energy intake and macronutrients.

Measures of activity related energy expenditure. Four different measures of activity were assessed from ADMR and BMR measurements. Firstly AEE was calculated using the formula 0.9 ADMR − BMR, correcting for 10% diet induced energy expenditure. Secondly, in order to correct for weight-bearing activities, AEE was divided by total body weight, leading to AEE/kg. Thirdly the physical activity level (PAL) was determined by dividing ADMR by BMR. Finally, the residual of ADMR (rADMR) was calculated from the regression of ADMR on BMR (Figure 1).

Statistical analysis. Differences between the independent variables of the PWS group and control group were analyzed by the two-sample *t*-test. Analysis of covariance was used to calculate the difference in ADMR between both groups, defined by the binary variable PWS, adjusted for bone age BMR, FM and gender as the other independent variables in the model. Firstly, the difference in regression slope of the influence of BMR on ADMR was tested using an interaction variable of PWS and BMR (PWS*BMR) adjusted for the variables in the model. Secondly, the difference between groups again adjusted for these independent variables, was estimated and tested for significance using linear regression assuming equal slopes. An analysis of covariance was also done for each of the measures of activity as the dependent variable, consequently using bone age, gender, FM and PWS as independent variables. The significance level was chosen at 5%. Data were expressed as means ± s.d. SPSS release 6.1 for Macintosh (SPSS Inc., Chicago, IL, USA) was used as the statistical package.

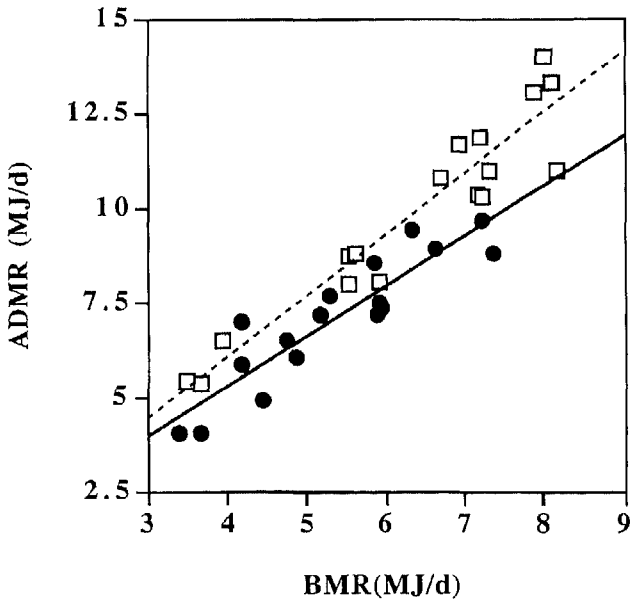

Figure 1 Average daily metabolic rate (ADMR, in MJ/day)as a function of basal metabolic rate (BMR, in MJ/day) plotted for the Prader–Willi group (PWS, in solid circles) and obese control group (controls, in open squares). The regression equation for PWS is: ADMR = 1.32 BMR + 0.06 ($r^2 = 0.83$); that for obese control is ADMR = 1.61 BMR − 0.34 ($r^2 = 0.90$).

Results

Energy expenditure and body composition

Clinical characteristics of PWS patients and controls are shown in Table 1. There were no statistically significant differences in age, height, weight, BMI, %RBW ((body weight/weight predicted by height and gender)*100) between both groups. FFM was smaller in the PWS group while FM and %FM were similar. FQ was also similar in both groups (PWS and controls: 0.87 ± 0.02).

BMR and ADMR as well as the measures of activity related energy expenditure were significantly lower in the PWS group compared with the controls (Table 2). BMR adjusted for weight (BMR_{Weight}) was lower in the PWS group, while BMR adjusted for FFM (BMR_{FFM}) was not different. Likewise, ADMR was adjusted for weight and also for FFM however, both calculations were lower in the PWS group compared with the controls ($P < 0.001$). ADMR was plotted against BMR in Figure 1. When ADMR was expressed as a function of BMR in separate linear regressions for the PWS group and the control group, the r^2 was 0.83 and 0.90, respectively. From a further

Table 2 Measures and calculations of energy expenditure

	PWS (n = 17)		*Obese controls (n = 17)*	
	Mean	s.d.	*Mean*	*s.d.*
BMR (MJ/day)	5.36*	1.18	6.38	1.55
BMR_{Weight} (MJ/day)	5.17*	1.57	6.57	1.92
BMR_{FFM} (MJ/day)	5.31	1.38	6.43	1.86
ADMR (MJ/day)	7.14**	1.72	9.94	2.64
$ADMR_{Weight}$ (MJ/day)	6.28***	2.60	10.80	3.12
$ADMR_{FFM}$ (MJ/day)	6.55***	2.02	10.54	3.04
AEE (MJ/day)	1.07***	0.69	2.56	1.03
AEE/kg (kJ/kg day)	23.11***	17.05	46.09	17.79
PAL	1.33***	0.15	1.55	0.12

PWS, Prader–Willi syndrome; BMR, basal metabolic rate; BMR_{Weight}, BMR adjusted for weight; BMR_{FFM}, BMR adjusted for fat-free mass; ADMR, average daily metabolic rate; $ADMR_{Weight}$, ADMR adjusted for weight; $ADMR_{FFM}$, ADMR adjusted for fat-free mass; AEE, activity induced energy expenditure; AEE/kg, AEE per kg body weight; PAL, physical activity level (ADMR/BMR).
Significantly different from control group (independent-samples *t*-test). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

linear regression model, it was evident that corrected for BMR, bone age, FM and gender, the difference in ADMR between PWS and controls was significant. The coefficients of BMR, gender and PWS significantly explained the variance of ADMR. Because the interaction variable PWS*BMR was not significant in the regression analysis, this variable was not included in the table (Table 3).

Measures of activity related energy expenditure

Table 4 presents the results of the difference between the PWS and control group for AEE, AEE/kg, PAL and rADMR adjusted for bone age, FM and gender. The variance of AEE was significantly explained by FM, gender and PWS. When AEE/kg or PAL was used as dependent variable, the coefficient of FM was not statistically significant. Only the coefficient of PWS significantly contributed to the variance of rADMR.

Table 3 Results of multiple-linear-regression analysis of the influence of basal metabolic rate (BMR), bone age, fat mass (FM), gender and Prader–Willi syndrome (PWS) on average daily metabolic rate (ADMR, in MJ/day)

Variable	*β coefficient*[a]	*s.e.*[b]	*95% CI β*[c]	*P*
BMR (MJ/day)	1.285	0.211	0.853–1.716	0.000
Bone age (y)	0.011	0.082	−0.157–0.179	0.892
FM (kg)	0.027	0.020	−0.015–0.069	0.193
Gender	−0.743	0.327	−1.414–−0.073	0.031
PWS[d]	−1.394	0.327	−2.064–0.725	0.000

[a]The partial regression coefficient, which is the change in ADMR for a change in a specific variable adjusted for the other independent variables in the equation.
[b]The standard error of the partial regression coefficient.
[c]The range of values that includes the population value of the coefficient, with 95% probability.
[d]Grouping variable PWS was defined: PWS = 1; controls = 0. The interaction variable PWS*BMR was not significant.

Table 4 Results of multiple-linear-regression analysis of the influence of bone age, fat mass (FM), gender and Prader–Willi syndrome (PWS) on measures of activity-related energy expenditure

Activity parameter[a]	*Variable*	*β coefficient*[b]	*s.e.*[c]	*P*
AEE (MJ/day)	Bone age (y)	0.046	0.060	0.451
	FM (kg)	0.033	0.015	0.042
	Gender	−0.785	0.259	0.005
	PWS[d]	−1.386	0.247	0.000
AEE/kg (KJ/(day*kg))	Bone age (y)	−1.515	1.167	0.204
	FM (kg)	−0.287	0.302	0.350
	Gender	−13.88	5.069	0.010
	PWS	−24.00	4.826	0.000
PAL	Bone age (y)	−0.001	0.011	0.933
	FM (kg)	0.002	0.003	0.510
	Gender	−0.121	0.046	0.015
	PWS	−0.218	0.044	0.000
rADMR (MJ/day)	Bone age (y)	−0.074	0.069	0.293
	FM (kg)	0.008	0.018	0.675
	Gender	−0.469	0.300	0.128
	PWS	−1.080	0.285	0.001

[a]AEE, activity induced energy expenditure, 0.9ADMR − BMR; AEE/kg, AEE per kg body weight; PAL, physical activity level, ADMR/BMR; rADMR, residual of ADMR on BMR.
[b]The partial regression coefficient which is the change in AEE for a change in a specific variable adjusted for the other independent variables in the equation.
[c] The standard error of the partial regression coefficient.
[d]Grouping variable PWS was defined; PWS = 1; controls = 0.

Discussion

The present study demonstrates a low activity related energy expenditure in children and adolescents with Prader–Willi syndrome as reflected in the following parameters: AEE, AEE/kg, PAL and rADMR. To control for the possible effects of body composition, biological maturity and gender on the measures of activity, each of the parameters was adjusted for bone age, fat mass (FM) and gender.

Daily physical activity can be divided into weight dependent and non-weight dependent activities. Although at present the doubly labelled water method functions as the gold standard to calculate the energy expended for activity in a free-living situation, the optimal adjustment for body weight as a correction for weight-bearing activities is still under debate.[13,21,22] The most direct method of measuring absolute activity related energy expenditure is by subtracting BMR from ADMR, correcting for 10% of diet induced energy expenditure: AEE = 0.9 ADMR − BMR. The present results show that the variation in AEE between subjects is not only caused by PWS but also by the differences in FM. Because at least part of the routine daily activities are weight-dependent, a higher FM will result in a higher energy cost performing the same, weight-dependent, tasks. In a study with prepubertal children,[23] FM did not correlate with AEE. This is possibly related to the energy efficiency physical activity, improves with maturation, which may lead to a higher towards the effect of FM on activity when age increases. Moreover, in an 2 earlier report by the same group, AEE was found to relate significantly with FM.[24]

AEE divided by total body weight[25,26] is an alternative approach assuming that all activity induced energy expenditure is related to weight-bearing activities. Previous studies, in children,[27,28] as well as adults[29,30] used this approach to demonstrate that obesity was associated with decreased levels of physical activity. Others[21,31–33] could not find an effect of obesity. In the present study, however, AEE/kg was not influenced by FM. Moreover FM could not take away the influence of PWS on AEE/kg indicating that adiposity cannot explain the decreased activity related energy expenditure in PWS, not even for weight-bearing activities. Since the majority of daily activities involve only limb movements and are not weight dependent, it has been suggested for sedentary adults to divide AEE by weight 0.5.[13] However, such an exponent is likely to be population specific. The amount and intensity of whole body movements during the day is probably related to age, gender

and especially to disease or disability. If such group-specific exponents for weight correction were determined, one of the general problems of using AEE as a measure of activity would remain the positive correlation with ADMR. As AEE will generally increase for higher values of ADMR, AEE as a measure of activity cannot be validly compared between groups with large differences in ADMR.

Correcting for metabolic body size by dividing ADMR by BMR is a way of losing the positive correlation between ADMR and BMR leading to the measurement of physical activity index or level (PAL). The results show that the subjects with the highest PAL are the boys in the control group. Other studies,[27,34,35] measuring PAL in adolescents did not show a gender difference in PAL during childhood through adolescence. Probably, the gender difference is caused by an average higher BMR in boys from the control group, as was previously demonstrated in obese adolescents.[27] An important assumption when comparing the PAL between groups is a constant relationship between ADMR and BMR. The positive intercept of the PWS group and the negative intercept of the control group (Figure 1) show that the assumption is incorrect. Although the gender difference in BMR within the control group was not significant, the combination with the negative intercepts of the control group will result in a higher PAL the boys by mathematical definition.[8,22] Therefore, the PAL is not the most appropriate way to compare groups with significant intercepts in the regression of ADMR on BMR, in spite of he similar ranges of BMR and ADMR in both study groups.

A valid technique suggested as an alternative approach to adjust data with non-zero intercepts is the analysis of covariance by multiple regression.[21] In this technique, the residuals from the regression of ADMR on BMR (rADMR) are itself a (relative) measure of the activity related energy expenditure corrected for BMR, assuming independence, a normal distribution of the data and a significant correlation between the dependent and independent variable. In the present study, BMR was the best single determinant of ADMR in the PWS as well as the control group, explaining respectively 90% and 83% of its variance, which was even higher than in other studies.[21,27] Interestingly, when rADMR is used as measure of activity, gender is no longer statistically significant, which indeed indicates that the gender difference in the previous measures of activity was in fact a BMR effect. Again, this approach points out a decrease in activity related energy expenditure in PWS patients.

One of the modulators for activity related energy expenditure is seasonality. Goran *et al*[36] showed that the AEE. and as a result also ADMR in prepubertal children was significantly higher in the spring compared with autumn, even when adjusted for body composition. In the present study the PWS and control subjects were all measured during the summer, avoiding this potential confounding factor. It is unclear whether seasonality has influenced the results of the other studies on energy metabolism in PWS, because the season of measurement was not mentioned in any of the reports. In the study by Schoeller *et al*,[5] the average ADMR of the PWS group was 53% of that of obese controls, which is lower than the 72% that was found in the present study. In Schoeller's study a standard RER of 0.85 was used to calculate ADMR, while in this report RER was equal to FQ, that was derived from a food record. Since FQ was similar in the PWS and control group it is unlikely hat this discrepancy in methods could help understand the group difference in ADMR. It is more likely that this is influenced by our younger study population. On the other hand, the average activity related energy expenditure was 60% lower for the PWS group in the present study, where Schoeller *et al* observed only a 40% reduction. The difference might be explained by the use of an equation derived by Ravussin *et al*[25] to calculate the ADMR, containing FFM, percentage activity and weight as explanatory variables. This equation was developed from respiration chamber measurements when in the present study ADMR was measured by doubly labelled water. It is likely that inter-individual differences in activity cannot be detected in a laboratory setting as easily in a free-living situation, which presumably resulted in a small coefficient for activity in Ravussin's formula.

Davies *et al*[11] measured PAL in a group of 10 children with PWS and compared them with a cohort of schoolchildren from an existing database. Although the authors were not able to detect a statistically significant difference in ADMR, and, given the small difference in the absolute outcome of ADMR, AEE and PAL, the results of the PWS subjects are remarkably similar. Therefore, as the authors suggested, the small study sample was probably the cause of the insignificant difference in ADMR between groups.

The cause of PWS children spending less energy on activity cannot be answered by the present study. The dysfunction of various hypothalamic systems, as one of the possible reasons for the low FFM in patients with this syndrome, might well be the underlying cause. The hypotonia in early childhood and under-development of muscle strength and coordination with delayed motor milestones may lead to a lack of capability and interest to be physically active. Additionally, the possible functional growth hormone deficiency and decreased levels of gonadotrophins[37] might take away the natural urge of children to be lively and playful.

In summary the present study demonstrates that AEE is lower in children and adolescents with PWS than in obese controls, matched for bone age and gender. In addition, all measures of activity related energy expenditure corrected for body size and adjusted for bone age, gender and FM, support the conclusion that PWS patients are less active during childhood and adolescence.

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